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IMPACT ASSESSMENT OF CREATINE ON MANY PHYSIOLOGICAL AND BIOCHEMICAL SCALES IN MALE RABBITS

Nawras A. ALWAN[1]

University of Basrah, Iraq

Eman Aboud AL-MASOUDI

University of Basrah, Iraq

Alia M. KUDAYER

University of Basrah, Iraq

Abstract

Creatine is a substance found naturally in body tissues, especially in the muscles and brain. Creatine is found in the diet, especially in seafood and red meat, and is commonly used to increase muscle mass and improve exercise performance in humans. This research was design to evaluate the effects of creatine administration on many physiological and biochemical parameters. In the method design, 24 mature adult male rabbits are divided at random into four groups as the following: G1 (Group of control): six mature adult of male rabbits orally administered distilled H2O (1ml/animal) by gavage daily, G2 mature adult of male rabbits were orally administration creatine monohydrate (1ml/kg BW) by gavage daily. G 3 mature adult of male rabbits were orally administration creatine monohydrate (2ml/kg BW) by gavage daily. While G4 adult male rabbits were orally administration creatine monohydrate (3ml/kg BW). After 30-days of administration, the rabbits were anaesthetized and blood samples were collected for determination thyroid hormones, liver enzymes, lipid profiles and kidney function (determined by urea, creatinine levels) and serum total protein levels, in the results, the groups that received 1, 2 and 3 ml of creatinine significantly increased but more over increased in group treated with 3 ml of creatinine in all studied parameters such as T4 hormones, liver enzymes, urea, creatinine levels and all lipid profile except TSH, T3, HDL, total protein concentration decreased significantly compared to the control group. In conclusions, the creatine has harmful effect on biochemical and physiological parameters in the creatine-treated group (3ml). In the conclusion that an adverse effect of creatine on the functions of the thyroid gland, liver and kidneys increases in these organs with increase doses.

Keywords: Creatine, Thyroid Hormones, AST, ALT, ALP, Male Rabbits.

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[1] nawras.alwan@uobasrah.edu.iq, https://orcid.org/0000-0001-8101-2545

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Introduction

Creatine (alpha-methyl of guandino-acetic acid) is derivative of amino acid synthesized from glycine, methionine and arginine in the pancreas, liver and kidneys. It is substance found normally in the tissues of body especially in the muscles and brain. Creatine is found in the diet especially in seafood and red meat and it is used commonly for increasing muscle mass and improving exercise performance in humans (Jäger *et al.*, 2011). It is important in making the energy for the muscles. Certain amount of creatine is only hold by muscle (skeletal muscles) in forms either the free (about 40 %) or phosphorylated (about 60%). The point of saturation of loading dose is usually reached within the first few days after taking. The main source of creatine in humans is production from endogenous or from consumption naturally in the diet. Exogenous supplemented of creatine, intra-muscular and stores in cerebral of creatine and its form of phosphorylated, phosphocreatine, creatine elevated. When increase the stores of creatine can offer therapeutically benefits by preventing diminishing of ATP, activating synthesis of protein or decreasing protein degradation and settle down of membranes (Joy *et al.*, 2014). About 1–2 g of creatine in humans is required to replace that lost by irreversible conversion to creatinine and about one-half of this is provided from endogenously synthesized and the remainder is from the diet (Poortmans *et al.*, 2010). Also creatine is taken orally for syndromes deficiency of creatine that influences the brain and obstructively chronic pulmonary disease. *Diabetes mellitus*, Congestive of heart failure (CHF), fibromyalgia, depression, inflammatory myopathies, disease of Parkinson, diseases of the muscles and nerves, sclerosis multiply, cramps and muscle atrophy, problems of breathing in infants, trauma in head, eye gyrate atrophy, schizophrenia, the senses and movement inherited disorders, breakdown of the spine muscle, and recovery from surgery (Gosselink, *et al.* 2003; Bender *et al.*, 2005; Freilinger *et al.*, 2011; Kley *et al.*, 2011; Carvalho *et al.*, 2012; Candow *et al.*, 2014). Carbohydrate interactions with creatine by carbohydrates combine with creatine can elevation creatine levels in muscle much more than creatine unique. Creatine in a dose 5 g supplementing plus 93 g of simply carbohydrates four doses/day for 5 days can increment creatine concentration 60% in muscle more than creatine unique (Koenig *et al.*, 2008). The current study aims for the examination the toxic effects of creatine in mature male rabbits.

Materials and Methods:

1- Animals of experimental:

This study was completed at the College of Veterinary Medicine/ University of Basrah. Twenty four adult male rabbits which were uses for this study. Kept of the animals done in the animal house to acclimatization fourteen days. Maintained the animals under optimum condition (25± 2) and (equally hours light to dark) cycle, with standard diet and tap water.

2- Experimental Design: The experimental animals were included in this study: twenty four adult male rabbits are randomly divided as the following into 4 groups:

G 1 (control): six adult mature male rabbits administered orally distilled water (1ml/kg BW daily) by gavage.

G 2: six adult mature male rabbits were administered orally creatine monohydrate (1ml/kg BW) dissolved in 5ml distilled water daily by gavage.

G 3: six adult mature male rabbits were administered orally creatine monohydrate (2ml/kg BW) dissolved in 5ml distilled water daily by gavage.

G 4: six adult mature male rabbits were administered orally creatine monohydrate (3ml/kg BW) dissolved in 5ml distilled water daily by gavage.

3- Studied parameters: After blood samples were collected from the heart in tubes without anticoagulant and samples of serum were isolated and stored it at -20 C until for biochemical examination.

3-1 Measurement of thyroid hormones concentrations (ng/dl):

The measurement of serum tetra- and trio- iodothyronine concentrations (T_4 and T_3) are generally regarded as a valuable tool in the diagnosis of thyroid dysfunction; kit was used (Monobind Inc. lake forest CA 92630, USA). The Procedure of the test was done according to that described by Ma *et al.* (2006).

3-2 Measurement of Thyroid-Stimulating Hormone (TSH) concentration (µlU/ml):

Serum thyrotropin (TSH) concentration measurement is regarded generally as a valuable tool in the diagnosis of thyroid dysfunction; kit was used (Monobind Inc. lake forest CA 92630, USA). The procedure of this test is similar to that described in T_4 and T_3 except adding TSH enzyme conjugate solution.

4- Biochemical measurements: Some biochemical measurements were done on the serum after the separation by using special enzymatic kits as follow:

4-1 Serum Aspartate Aminotransferase (AST) Serum Alanine Aminotransferase (ALT) estimations (U/I): Aspartate and alanine aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine . The procedure of this kit was mentioned by Schumann and Klauke (2003). From a table in the paper of kit the activity of AST and ALT in the serum can be obtained by plotting the measured absorbanceagainst the transaminase activities in U/I.

4-2 Serum Alkaline Phosphatase (ALP) estimation (U/I):

This calculation of ALP concentration was done by using the colorimetric determination of alkaline phosphatase activity (Biomeriexu, France). The procedure could be an estimation of ALP activity by methodology was described by Tietiz (1999).

Calculation:

$$\textbf{ALP (U/I)} = \frac{\text{OD Serum sample - OD seum blank}}{\text{OD standard}} \textbf{ X 142 U/I}$$

4-3 Total cholesterol measurement (TC): The serum total cholesterol was measured enzymatic by using a linear chemical kit (BIO-ABO S.A/CHOD-PAP, France. The measurement mentioned by Tietz (1996 &1999).

Total cholesterol concentration was calculated by following equation:

$$\textbf{Total Cholesterol concentration} = \frac{A\ samle}{A\ sample} \times 200\ \textbf{mg/dl}$$

4-4 measurement of triglyceride in serum (TG): The measurement of serum triglyceride concentration was done by using a special chemical kit (SYRBIO/GPO-PAP/ Syria) based on Stein (1987). Calculated the serum TG concentration by the following formula: TG

Concentration of TG $= \frac{A\ sample}{A\ sample} \times 200\ mg/dl\ of\ blood$

4-5 measurement of concentration of High-Density Lipoprotein Cholesterol (HDL-C):

This measurement was described by Tietz (1999). And concentration it can be obtained by application the following formula:

HDL concentration $= \frac{A\ sample}{A\ sample} \times standard\ concentration \times 1.1$

4-6 Measurement of Low-Density Lipoprotein Cholesterol in serum (LDL-C):

Concentration of serum LDL can be calculated by the following equation (Ram, 1996).

LDL = TC – (HDL+TG/5)

4-7 Measurement of Serum very Low-Density Lipoprotein (VLDL): Serum VLDL concentration was calculated by dividing serum TG/5 (Firedwald *et al.*, 1972).

Results: 1- Creatine effect on hormones of thyroid concentrations:

The results tablets in table (1) appeared significantly ($p< 0.05$) elevation in serum TSH concentrations and a decrease significant ($p< 0.05$) in serum T_4 and T_3 in creatine-treated groups (G3 and G4) as compared to the group of control (G1).

Table (1) Creatine effect on serum concentrations of TSH, T_4 and T_3 in mature adult of male rabbits:

Parameters / Groups	TSH µIU/ml	T_4 µg/dl	T_3 ng/dl
G 1(Control group)	8.27 ±1.74 a	9.83 ±0.86 a	2.13 ±1.37 a
G 2(Creatine 1gm)	8.12 ±2.25 a	9.27 ±1.27 a	2.16 ±0.57 a
G 3(Creatine 2gm)	7.46 ±2.21 b	8.66 ±1.34 b	2.61 ±0.82 b
G 4(Creatine 3gm)	7.01 ±1.99 b	7.15 ±1.05 b	2.80 ±0.44 b
LSD	1.10	0.66	0.8

Small letters values denote to mean significant differences at ($p< 0.05$ levels) (M±SD),(no. =10).

2- Creatine effect of on ALT, AST and ALP concentrations:

The creatine-treatment effect for 30 days on serum concentrations of ALT, AST and ALP are noticed in table (2). The data was indicated to a significant ($p< 0.05$) increased in serum concentrations of ALT, AST and ALP in groups of creatine-treated compared with control.

Table (2) creatine effect on serum concentrations of ALT, AST and ALP in mature adult of male rabbits:

Parameters / Groups	ALT (U/l)	AST (U/l)	ALP (U/l)
G 1(Control group)	17.80± 4.00 a	21.98±2.10 a	11.44±0.72 a
G 2(Creatine 1gm)	18.38±3.37b	22.00± 3.41a	11.77± 1. 04a
G 3(Creatine 2gm)	19.95± 4.98c	22.17± 3.04 a	12.00± 0.47 b
G 4(Creatine 3gm)	22. 53± 2.65d	25.25± 3.60 b	14.14± 0.63c
LSD	0.63	0.96	0.45

Small letters values denote to mean significant differences at (p< 0.05 levels) (M±SD),(no. =6).

3- Creatine effect on profile of lipid in mature adult of male rabbits:

The data of results in table (3) showed a significant increment in total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) concentrations in creatine groups as compared to the control group. While a significantly decrease (p< 0.05) in HDL concentration was appeared in creatine administration groups compared to the G1.

Table (3) Creatine effect of on serum lipid profile in adult male rabbits:

Parameters / Groups	TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
G 1(Control group)	82.28 ±4.59 a	81.74 ±6.39 a	54.97 ±3.59 a	20.17 ±4.10 a	15.88 ±1.66 a
G 2(Creatine 1gm)	87.90 ±6.57ab	75.98 ±8.31 ab	50.27 ±5.69 b	22.24 ±4.62 b	16.59 ±0.75 a
G 3(Creatine 2gm)	95.42 ±8.08 b	80.50±14.03 ab	47.58 ±7.56 b	25.21 ±8.72 c	18.41 ±2.29 b
G 4(Creatine 3gm)	113.97±8.63 c	97.40±0.78c	40.60±14.62c	26.70±1.58 c	24.89 ±4.70 c
LSD	14. 58	49.93	3.78	9.89	2.44

Small letters values denote to mean significant differences at (p< 0.05 levels) (M±SD),(no. =6).

4- Creatine effect on serum TP, urea and creatinine concentrations in mature adult of male rabbits: Creatine treated groups appeared increase significantly (p<0.05) in serum urea (Ure.) and creatinine (Creat.) concentrations compared with control group (Table 4). While the concentration of serum total protein decrement statistically (p<0.05) in groups of creatine administration than to the group of control.

Table (4) Creatine effect on serum concentrations of TP, Ure. and Creat. in mature adult of male rabbits:

Parameters / Groups	TP g/L	Ure. mg/L	Creat. mg/dl
G 1(Control group)	8.39±0.51[a]	62.39±7.86 [a]	2.72±0.64[d]
G 2(Creatine 1gm)	7.72±0.63[b]	76.01±3.93[b]	4.23±0.50[c]
G 3(Creatine 2gm)	7.36±0.61[b]	84.32±4.59[c]	5.47±1.04[b]
G 4(Creatine 3gm)	7.33±0.82[b]	107.72±11.43[d]	6.84±0.47[a]
LSD	1.08	12.21	1.43

Small letters values denote to mean significant differences at (p< 0.05 levels) (M±SD),(no. =10).

Discussion:

The current study showed a statistically decrease in concentrations of in T_3 and T_4 is seen in creatine treated animals than to group of control. The explanation of these result due to that creatine caused a disturbance in synthesis of thyroid hormones in the thyroid gland or metabolism that leads to decreased concentrations of thyroid hormone with an increase in TSH hormone. The depletion of thyroid hormone concentrations in creatine – treated group may be resulted from iodine deficiency that lead to failure of thyroid gland to synthesize thyroid hormones and resulted into hypothyroidism .

The result appeared in (table 2), revealed elevations in plasma levels of liver enzymes (AST, ALT) and alkaline phosphate (ALP) in groups treated with creatine as compared with to the control group may be attributed to the increased permeability of hepatocytes cell membrane or to liver tissue damage and leakage of enzymes to the extracellular fluid. These results are agreed with that of Boada *et al.* (1999) and McGill (2016) who reported that plasma levels of hepatic enzymes; AST, ALT and ALP which are valuable considerably for toxic effects detecting in the liver.

Table 4 revealed a significant increase of Ure. and Creat. are regarded as markers classically for renal function due to they represent a marker simply for filtration of glomerular (Ghosh and Sil, 2007). It is also showed a statistically decrement in serum total protein concentrations in the creatine-treated mature male rabbits as compared with the Control. The results of this study revealed the adverse effects of creatine on the biochemical and physiological parameters as similar recorded by Al-Masoudi *et al.* (2021) on the body weights and reproductive parameters.

The literature still discusses creatine as a dietary supplement, albeit less frequently (De Souza e Silva *et al.*, 2019; Antonio *et al.*, 2020; Clarke *et al.*, 2020). Some studies have reported that the compound can have adverse effects on Kidneys function (Thorsteinsdottir, 2006; Taner *et al.*, 2011). On the other hand, other researchers have not determined the risk of renal damage, and episodes of nephrotoxicity are rare in healthy patients supplemented with Creatine (Gualano *et al.*, 2008; Gualano *et al.*, 2011; Domingues *et al.*, 2020).

Conclusions:

The adverse effect of creatine on biochemical and hormonal parameters more in the group treated with creatine. Also the adverse effect of creatine on thyroid, liver and kidney functions in these organs more in the fourth group.

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